John von Neumann Institute for Computing

Computer Simulations of Product Dissociation from the Active Site of the Anthrax Edema Factor

L. Martínez, Th. Malliavin, M. Nilges, A. Blondel

published in

From Computational Biophysics to Systems Biology (CBSB08),
Proceedings of the NIC Workshop 2008,
Ulrich H. E. Hansmann, Jan H. Meinke, Sandipan Mohanty,
Walter Nadler, Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **40**, ISBN 978-3-9810843-6-8, pp. 317-320, 2008.

© 2008 by John von Neumann Institute for Computing
Permission to make digital or hard copies of portions of this work for personal or classroom use is granted provided that the copies are not made or distributed for profit or commercial advantage and that copies bear this notice and the full citation on the first page. To copy otherwise requires prior specific permission by the publisher mentioned above.

http://www.fz-juelich.de/nic-series/volume40

Computer Simulations of Product Dissociation from the Active Site of the Anthrax Edema Factor

Leandro Martínez, Thérèse Malliavin, Michael Nilges, and Arnaud Blondel

Unité de Bioinformatique Structurale, Institut Pasteur and URA CNRS 2185,
28, rue du Dr. Roux, 75015 Paris, France
E-mail: leandro@pasteur.fr

The Anthrax Edema Factor is an adenylyl cyclase responsible for the overproduction of cyclic mono-phosphate (cAMP) from ATP causing host cell deregulation. For optimal catalytic activity, it should efficiently release the reaction products, cAMP and Pyrophosphate. Here we study the mechanisms cAMP and PPi dissociation using Locally Enhanced Sampling and Steered Molecular Dynamics simulations. Since there is no clear consensus on the number of metal ions in the catalytic site, simulations were performed in the presence of one or two cations. The simulations suggested that the presence of the second metal ion greatly impairs product dissociation supporting the hypothesis of an optimal one-ion catalytic binding site.

1 Introduction

Anthrax bacteria produce three major toxins: Protective Antigen, Lethal Factor and Edema Factor (EF). EF enters the cell bound to the Protective Antigen. Once released in the cytoplasm it binds Calmodulin (CaM).[1] Then, a large conformational change[1] activates the adenylyl cyclase function of EF which converts ATP to cyclic-AMP (cAMP) and Pyrophosphate (PPi). To efficiently catalyse the cyclization of ATP, EF must bind ATP, stabilize the transition state (TS) and rapidly release the reaction products. TS stabilization depends on the binding of Mg^{2+} ions to the catalytic site.[2,3] The crystal structures of the EF-CaM complex bound to reaction products (1SK6[3]), contains Yb^{3+} in the active site. Two Yb^{3+} binding modes are observed: an one-ion binding mode and a two-ion binding mode, which are illustrated in Figure 1. Therefore, it is not clear whether the reaction proceeds in the presence of one or two ions. The energetic balance between TS stabilization and facilitated product release must be understood to evaluate which is the optimal active site arrangement. Here, a study combining Locally Enhanced Sampling (LES)[4] and

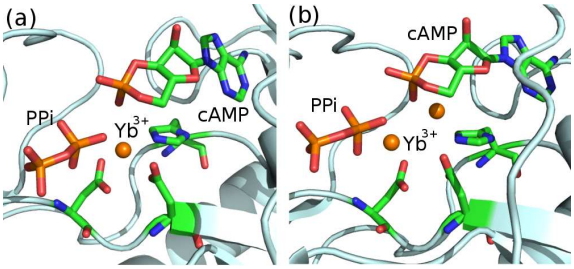

Figure 1. Ionic (Yb^{3+}) binding modes observed in the crystallographic structure of EF bound to reaction products:[3] (a) One-ion and (b) two-ion binding modes.

Steered Molecular Dynamics simulations (SMD)[5] was used to investigate the dissociation mechanisms of PPi and cAMP from the active site of the Anthrax Edema Factor under different Mg^{2+} contents. PPi and cAMP dissociated through different solvent-accessible cavities. The Mg^{2+} content of the active site greatly affected the forces required to induce product dissociation, indicating that a one-metal ion binding site would be more favorable for efficient product dissociation.

2 Molecular Dynamics Simulations of Product Dissociation

LES simulations, performed with CHARMM,[6] were used to observe dissociation of the reaction products without *a priori* assumptions on the mechanisms.[4] In this case, multiple copies of either PPi or cAMP are placed in the active site. The interaction of each copy with the protein is inversely proportional to the number of copies, and the copies do not interact with each other. We have performed 500 ps simulations with 1 to 60 copies of each of the reaction products. LES simulations were done in vacuum starting from the structure 1SK6,[3] for both ion binding modes. The ions Yb^{3+} were replaced by Mg^{2+}.

Dissociation was observed at a minimal level of 28 PPi copies. A similar number of PPi dissociation events was observed: 66 for the one-Mg^{2+} and 67 for the two-Mg^{2+} binding mode in all simulations. Dissociation of cAMP was observed with smaller number (four) of copies, and if more than 18 copies are present, all cAMP dissociated in all runs. The number of cAMP dissociation events observed for the one-Mg^{2+} binding mode was slightly larger than for the two-Mg^{2+} binding mode (77 and 61 respectively, for simulations up to 18 copies).

As shown in Figure 2, there are two discernible solvent-accessible cavities in each side of the protein Each product dissociated roughly in the directions indicated by these cavities, however displaying a significant dispersion of dissociation angles. The details of the dissociation along these directions were studied by SMD simulations.

In SMD an external force is applied to probe how difficult it is to induce ligand dissociation.[5] Simulations were performed with NAMD[7] for the fully solvated 1SK6 system, prepared with Packmol.[8] The force profiles for PPi and cAMP dissociations were (Figure 3) significantly different in in each Mg^{2+}binding mode. The presence of two ions in the active site greatly impairs product dissociation. The forces inducing product release increase from about 1500 to 3900 pN for PPi, and from 950 to 2400 pN for cAMP.

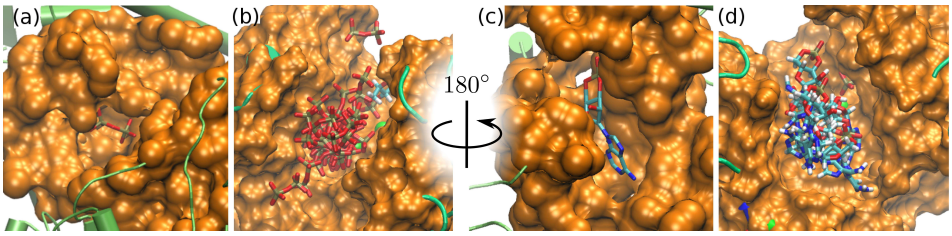

Figure 2. Product dissociation observed in LES simulations: (a) PPi solvent accessible cavity and (b) PPi dissociation. (c) cAMP solvent accessible cavity and (d) cAMP dissociation. The solvent accessible cavities are in opposite sides of the protein.

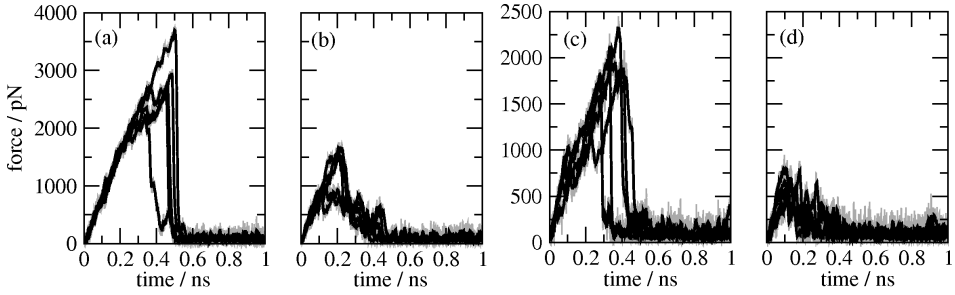

Figure 3. Forces leading to dissociation of PPi (a,b) and cAMP (c,d): five MD runs for each system where performed with variable pulling directions. The force moduli are plotted in presence of one- (a,c) and two-Mg^{2+} (b,d).

3 Concluding Remarks

The simulations suggest that the product dissociation is easier in presence of one-ion in the active site. This is in agreement with the decrease in catalytic activity observed experimentally for large Mg^{2+} concentrations[3] and suggests that the one-Mg^{2+} binding mode for EF is optimal for product release.

Acknowledgments

L. M. thanks CAPES (Brazil) for financial support.

References

1. C. L. Drum *et al. Structural basis for the activation of anthrax adenylyl cyclase exotoxin by calmodulin.* Nature **415**, 396-402, 2002.
2. Q. Shen *et al. Calcium-independent calmodulin binding and two-metal-ion catalytic mechanism of anthrax edema factor.* EMBO J. **24**, 929-941, 2005.
3. Q. Guo *et al. Structural and kinetic analyses of the interaction of anthrax adenylyl cyclase toxin with reaction products cAMP and pyrophosphate.* J. Biol. Chem. **279**, 29427-29435, 2004.
4. R. Elber, M. Karplus, *Enhanced sampling in molecular dynamics: use of time-dependent Hartree approximation for a simulation of carbon monoxide diffusion through myoglobin.* J. Am. Chem. Soc. **112**, 9161-9175, 1990.
5. B. Isralewitz *et al. Steered molecular dynamics investigations of protein function.* J. Mol. Graph. **19**, 13-25, 2001.
6. B. R. Brooks *et al. CHARMM - A program for macromolecular energy, minimization and dynamics calculations.* J. Comp. Chem. **4**, 187-217, 1983.
7. J. C. Phillips *et al. Scalable molecular dynamics with NAMD.* J. Comp. Chem. **26**, 1781-1802, 2005.
8. J. M. Martínez and L. Martínez, *Packing optimization for automated generation of complex system's initial configurations for molecular dynamics and docking.* J. Comp. Chem. **24**, 819-825, 2003.